

Novel Withanolides from the Flowers of *Datura tatula*¹

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Abstract – Three new withanolides, designated as withatatulins B, C, and D, were isolated from the fresh flowers of *Datura tatula* Linn. Detailed spectral analysis of these compounds permitted advancement of their structures respectively, as 5 β , 6 β -epoxy-12 β ,21-dihydroxy-1-oxo-witha-2,24-dienolide (2), 6 β ,12 β ,21-trihydroxy-1-oxo-witha 2, 4,24-trienolide (3) and 5 β ,6 β ,12 β ,21-tetrahydroxy-1-oxo-witha-2,24-dienolide (4a). Withanolides with oxygen functions both at 12 and 21-positions are rare and first reported from *Datura* species.

Key words – *Datura tatula*, Solanaceae, Withanolides, Withataulins B, C, and D, 12,21-Dioxygenated withanolides.

Introduction

Withasteroids are a growing group of naturally occurring steroidal lactones built on an intact or rearranged ergostane framework, and their distribution is restricted almost exclusively to the members of the family, Solanaceae. Of the different types of withasteroids, classified on the basis of their structural features, withanolides are most abundant in nature and these are compounds built on 22-hydroxy-ergostan-26-oic acid 26, 22-lactone skeleton (Ray and Gupta, 1994). *Datura* withasteroids belong to the class of withanolides many of which are oxygenated at C-21, a distinctive feature which was first witnessed by us in withametelin (1), isolated from *Datura metel* (Oshima *et al.*, 1987).

In continuation of our investigation on *Datura* withanolides (Manickam *et al.*, 1994), we present here the structure elucidation of three new withanolides, isolated from the

fresh flowers of *Datura tatula* Duthie, J.F., 1960), a plant the leaves of which yielded several known and a new 21-hydroxy withanolide, named withatatulins (Manickam *et al.*, 1996). Systematic fractionation of the methanolic extract of the fresh flowers of *D. tatula* and chromatographic resolution of the appropriate fractions yielded three new withanolides which were named withatatulins B, C, and D. Structural clarification of these compounds was made almost exclusively from spectral evidence.

Experimental

MPs were determined in open capillary and are uncorrected. UV spectra were recorded in MeOH on a JASCO UV-vis 7800 instrument. ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were taken with a Bruker AMX 400 spectrometer using CDCl₃ solutions unless otherwise stated. CC and TLC were carried

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out respectively, over silica gel (60-120 mesh) and silica gel G (Qualigens Fine Chemicals). C_6H_6 -EtOAc (1:1) and MeOH- $CHCl_3$ (1:9) were used for developing TLC plates.

Plant material - *Datura tatula* Linn. (Solanaceae) which grows in the sub-Himalayan tracts of India is often cultivated in gardens due to its large purple flowers with three layers of petals. The plants used in the present investigation were raised from the seeds collected from the garden of Chunar Fort, Mirzapur, Uttar Pradesh, India. The plant was identified by Professor G.N. Choudhury, Department of Botany, Banaras Hindu University. A voucher specimen of the plant is being kept in the department.

Extraction and isolation of withanolides from the fresh flowers of *Datura tatula* Linn - The freshly collected flowers (4 kg) of *D. tatula* were crushed and kept soaked in MeOH in a percolator for 7 days. The extraction was repeated twice, the combined methanolic extract was concentrated under reduced pressure and the resulting thick extract was diluted with water and subsequently extracted with EtOAc. The EtOAc-soluble fraction (20 g) was chromatographed over silica gel and eluted initially with hexane and then with hexane-EtOAc mixtures of increasing polarity. The fractions eluted with hexane-EtOAc (1:1) provided a gummy residue (1.8 g) which yielded **2**, **3**, and **4a** on rechromatography over silica gel and elution with hexane-EtOAc mixtures.

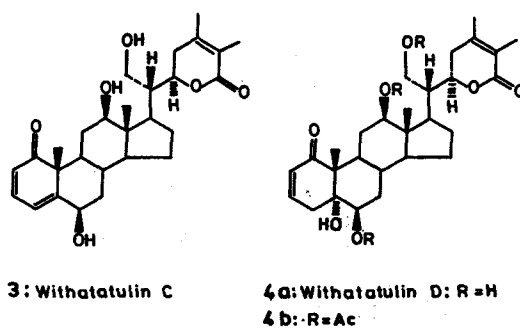
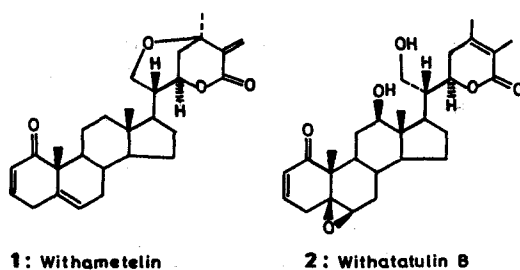
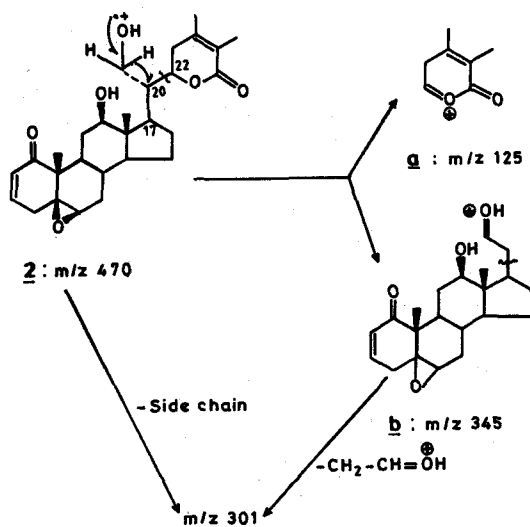
Withatatulins B (2) : Elution with hexane-EtOAc (1:1) yielded a solid (55 mg) which crystallized from EtOAc as white microcrystalline powder, m.p. 290-292 °C; UV λ_{max}^{MeOH} : 225 (ϵ , 12500); FABMS : m/z 471 (M+H)⁺, 493 (M+Na)⁺; EIMS : m/z 470 (16%), 345 (25%), 301 (16%), 147 (16%), 135 (17%), 125 (100%), 43 (85%). ¹H and ¹³C-NMR : see Table 1.

Withatatulins C (3): Elution with hexane-EtOAc (3:1) yielded a solid (35 mg) which crystallized from EtOAc as white needles, m.p. 240-242 °C; UV : λ_{max}^{MeOH} 312, 222 nm (ϵ 5000,

13000); EIMS : m/z 470; 125 (base peak); ¹H and ¹³C-NMR see Table 1.

Withatatulins D (4a) : Elution with hexane-EtOAc (1:3) yielded a solid (40 mg) which crystallized from EtOAc-MeOH as white needles, m.p. 242-243 °C; UV : λ_{max}^{MeOH} 222 nm (ϵ 14000); FABMS : m/z 489 (M+H)⁺; 511 (M+Na)⁺.

Withatatulins D triacetate (4b) - Withatatu-



lin D (25 mg) was acetylated in the usual way with $\text{Ac}_2\text{O}-\text{C}_5\text{H}_5\text{N}$ at ambient temperature and purified by column chromatography to yield an amorphous powder (20 mg); $^1\text{H-NMR}$ (400 MHz, CDCl_3), δ 6.54 (1H, *ddd*, $J=10.2$, 5.12, 2.28 Hz, H-3), 5.89 (1H, *dd*, $J=10.1$, 2.36 Hz H-2), 4.80 (1H, *t*, $J=2.64$ Hz, H-6), 4.75 (1H, *dd*, $J=10.96$, 4.48 Hz, H-12), 4.41 (1H, *dt*, $J=12.84$, 3.48, Hz, H-22), 4.36 (1H, *dd*, $J=11.58$, 6.8 Hz, H_a-21) 4.06 (1H, *dd*, $J=11.50$, 5.2 Hz, H_b-21), 1.92, 1.86, 1.24, 0.88 (3H, *s*, each Me-28, -27, -19,-18), 1.99, 2.00, 2.12 ($3 \times \text{OAc}$), found C, 66.12, H, 7.61%, $\text{C}_{34}\text{H}_{46}\text{O}_{10}$ requires C, 66.45, H, 7.49%.

Results and Discussion

Withatutulin B, $\text{C}_{28}\text{H}_{38}\text{O}_6$ (MH^+ , m/z 471), m.p. 290-292 °C, was recognized to be a typical withanolide from its diagnostic spectral features. On electron impact in a mass spectrometer, it underwent a facile cleavage into two halves by the scission across C-20-C-22 bond and showed the base peak at m/z 125 (ion a) and a significant peak at m/z 345 (ion b) (Scheme 1). The $^1\text{H-NMR}$ spectrum of withatutulin B showed the characteristic double triplet at δ 4.44 (1H, *dt*, $J=10.04$, 3.12 Hz), the signal for the oxymethine hydrogen

Table 1. NMR spectral assignments for compounds **2**, **3** and **4a**.

Position	$^1\text{H} \delta$ (m, J) (400 MHz)	^{13}C (ppm) (100 MHz)	$^1\text{H} \delta$ (m, J) (400 MHz)	^{13}C (ppm) (100 MHz)	$^1\text{H} \delta$ (m, J) (400 MHz)
	Withatutulin B(2)		Withatutulin C(3) ^a		Withatutulin D(4a) ^b
1	-	203.61	-	206.35	-
2	6.02(dd,10,2.6)	129.11	6.03(dd,10,0.8)	117.65	5.78(dd,10.1,2.5)
3	6.88(ddd,10,6,2,2.2)	145.11	7.04(dd,10,6)	141.18	6.66(ddd,10.1,5.1,2.2)
4	2.98(dt,19,2.7)[4 β]	33.29	6.20(dd,6,0.8)	125.72	3.28(dt,19.7,2.8)[4 β]
5	-	61.82	-	158.39	-
6	3.14(d,2.3)	63.40	4.54(t,2.8)	72.84	3.52(t,2.8)
7	-	30.85	-	39.71	-
8	-	28.79	-	31.91	-
9	-	43.28	-	47.69	-
10	-	47.80	-	53.61	-
11	-	32.77	-	30.07	-
12	3.50(dd,11.2,4.1)	78.08	3.39(dd,11.2,4.3)	77.98	3.57(dd,11.2,4.1)
13	-	48.00	-	47.88	-
14	-	54.31	-	53.65	-
15	-	23.77	-	23.82	-
16	-	28.68	-	28.45	-
17	-	46.22	-	46.26	-
18	0.76(2)	7.29	0.87(s)	7.22	0.82(s)
19	1.25(s)	15.03	1.49(s)	18.54	1.31(s)
20	-	46.50	-	46.38	-
21	3.92(dd,11.7,3.1) 3.97(dd,11.7,3.4)	58.71	3.88(dd,12.1,2.5) 3.95(dd,12.1,3.5)	58.41	3.84(dd,11.8,3.2) 3.90(dd,11.8,3.5)
22	4.44(dt,10,3.1)	78.28	4.49*dt,13.4,3.4)	78.08	4.51(dt,13.5,3.7)
23	-	32.29	-	29.40	-
24	-	150.41	-	151.16	-
25	-	121.49	-	121.08	-
26	-	167.26	-	167.83	-
27	1.84(s)	12.41	1.87(s)	11.92	1.89(s)
28	1.97(s)	20.45	1.96(s)	20.09	1.97(s)

m=multiplicity of signals, a=in $\text{CDCl}_3+\text{CD}_3\text{OD}$, b=in CD_3OD

(H-22) of the conjugated δ -lactone moiety which is often regarded as withanolide 'fingerprint' [(Ray and Gupta, 1994). The spectrum also showed signals for four methyl groups [two angular methyls (δ 0.76, 1.25, 3H, *s* each) and two vinylic methyl groups (δ 1.84, 1.97, 3H, *s* each)] associated with the α,β -unsaturated- δ -lactone. An additional methyl (Me-21) signal, discerned in the $^1\text{H-NMR}$ spectrum of a typical withanolide as a doublet, was, however, missing in the spectrum of withatatulins B and in its place an ABX pattern was discernible; the geminally coupled AB protons appeared as a pair of double doublets at δ 3.92 and 3.97 and the X proton as a multiplet around δ 2.09.

The pair of double doublets sharpened on D_2O exchange and this indicated C-21 to be present as a hydroxymethyl ($-\text{CH}_2\text{OH}$) group. The genesis of the mass peak at m/z 301 by elimination of a $\text{C}_2\text{H}_4\text{O}$ unit from ion **b** also corroborated the above assumption.

Of the six oxygen atoms present in the molecule of withatatulins B, two are involved in carbonyls associated with enone (δ_c 203.6) and α,β -unsaturated- δ -lactone (δ_c 167.2). For the remaining four oxygen atoms, signals for five oxycarbon signals (δ_c 58.7, 61.8, 63.4, 78.1, 78.3) are discernible in the $^{13}\text{C-NMR}$ spectrum of the compound which clearly indicates the presence of a cyclic ether, probably as an epoxide function. In fact, the resonance signals of the hydrogens and carbons associated with a steroidal $5\beta,6\beta$ -epoxy-2-en-1-one moiety were discernible in the NMR spectra of the compound, and these were found to match perfectly with those reported for withanolides having such an AB ring substitution pattern (Habtemariam *et al.*, 1993). In addition to the signals mentioned so far, $^1\text{H-NMR}$ spectrum of withatatulins B showed a characteristic double doublet at δ 3.50 ($J=11.16, 4.08$ Hz) assignable to an axial carbonyl hydrogen coupling with the neighbouring methylene hydrogens. This observation taken in conjunction with the high

field shift of 18-methyl carbon (δ_c 7.29) led to the placement of a β -hydroxyl group at C-12 (Evans *et al.*, 1984; Gupta *et al.*, 1992) and the structure of withatatulins B was advanced as $5\beta,6\beta$ -epoxy-12 β ,21-dihydroxy-1-oxowitha-2,24-dienolide (**2**) which was found to be in agreement with its $^{13}\text{C-NMR}$ data (Table 1).

Withatatulins C, $\text{C}_{28}\text{H}_{38}\text{O}_6$ (M^+ , m/z 470), m.p. 240-242 °C showed striking resemblance with **2** in spectral properties and it could be readily inferred that the two molecules had identical CD rings and the side chain. The UV spectrum of withatatulins C was, however, different from withatatulins B and the presence of a steroidal 2,4-dien-1-one chromophore was indicated from its absorption characteristics (λ_{max} 312, 222 nm). This was confirmed from its $^1\text{H-NMR}$ spectrum which showed signals for three contiguous olefinic hydrogens of a conjugated dienone (δ 6.03, 1H, *dd*; 6.20, 1H, *dd*; 7.04, 1H, *dd*). A survey of literature on withasteroids reveals that such a chromophore is invariably accompanied by a hydroxyl group at C-6 and while its presence can be readily detected from a low-field carbonyl hydrogen signal, its orientation can be decided from the chemical shift of 19-methyl group (Ishiguro *et al.*, 1975; Sahai *et al.*, 1982). In withatatulins C, H-6 appeared as a narrow triplet at δ 4.54 and 19-methyl as a singlet at δ 1.49 which unambiguously settled the β -orientation of the hydroxyl group at C-6. Withatatulins C was thus formulated as $6\beta,12\beta,21$ -trihydroxy-1-oxowitha-2,4,24-trienolide (**3**), a structure that received support from its $^{13}\text{C-NMR}$ data (Table 1).

Withatatulins D, $\text{C}_{28}\text{H}_{40}\text{O}_7$ (MH^+ , m/z 489), m.p. 242-243 °C was recognized to be a close relative of withatatulins B (**2**) and a difference only in the AB ring substitution pattern of the two molecules became manifest from a comparison of their spectral data. The difference in the molecular formulae of the two compounds and their double bond equivalence led to the speculation that witha-

tatulin D bears a vicinal trans-diol system in place of an oxirane ring present in **2**. This assumption gained credence from the observation that acetylation (Ac₂O/py) of withatulin D at room temperature yielded a triacetate (**4b**) and caused downfield shift of ¹H-NMR signals of four carbinylic hydrogens—two geminally coupled hydrogens of a primary alcohol and two methine hydrogens of two secondary alcoholic groups. While the double doublet at δ 3.57 (J=11.2, 4.12 Hz) which shifted to δ 4.75 spoke of the α-axial hydrogen at C-12, the narrow triplet at δ 3.52 (J=2.8 Hz) shifting to δ 4.80 suggested an equatorial hydrogen at C-6. In fact, the resonance signals of the hydrogens associated with AB rings of the molecule were in perfect accord with those of the corresponding hydrogens of withametelin G, a withanolide with a 5α,6β-dihydroxy-2-en-1-one moiety (Farboodniay Jahromi *et al.*, 1993). The structure of withatulin D was thus advanced as **4a**.

Both 12-hydroxy and 21-hydroxy withanolides have been reported earlier from *Datura* species but the three withanolides described above share the unusual feature of having hydroxyl groups at both these sites. Only the recently reported jaborosalactones R, S, and T which have a hemiketal or ketal ring involving C-12 and C-21 bear some resemblance with these withatatulins (Bonetto *et al.*, 1995).

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